Supercritical CO_2 Extraction of Carotene and Lutein from Leaf Protein Concentrates

FABIO FAVATI, JERRY W. KING, JOHN P. FRIEDRICH, and KENNETH ESKINS

ABSTRACT

Supercritical fluid carbon dioxide was used to extract carotene and lutein from leaf protein concentrate (LPC). Extractions were performed using pressures of 10–70 MPa at 40°C and CO_2 flow rates of 5–6 L/min. Over 90% of the carotene contained in LPC was removed at extraction pressures in excess of 30 MPa. Removal of lutein from LPC required higher extraction pressures (70 MPa) and gas volumes to attain a 70% recovery level. Experimental results were rationalized with the aid of solubility parameter theory. The described process offers the possibility of obtaining a selective extraction of natural colorants, free of solvent residuals, which can be used as food dyes.

INTRODUCTION

THE USE OF ARTIFICIAL DYES is a common practice in the modern food industry, but there is growing concern about their actual or potential effect on human health. This concern has led to an increasing interest and utilization of natural products as alternative food colorants. Carotenoids are one of the major groups of natural pigments that find widespread utilization in the food industry, in particular β-carotene. The addition of β-carotene to food serves two purposes: (1) impartation of color and (2) nutritional value, since it is a precursor of vitamin A. Furthermore, the role of carotenoids as longevity determinants in mammalian species has also been suggested (Cutler, 1984; Rettura et al., 1982; Seifter et al., 1982, 1983).

Traditional natural sources of carotenoids are the fruit of Bixa orellana, paprika, carrots; however, a large portion of commercially available β-carotene is also synthetically produced. Concerns regarding the effect of artificial dyes on human health suggest an expanding economic market potential for natural pigments, and a need for additional research into alternative natural substrates for the production of carotenoids. A key step in the production of alternative sources of carotenoids is the development of new extraction techniques, which can minimize molecular alteration of the carotenoids during the extraction process.

Leaf protein concentrates (LPC) were chosen as substrate for the extraction of these pigments. LPC has not only a high protein content but also an appropriate amino acid composition for use as a source of good quality protein for enhancing human nutrition. Therefore, much research has been carried out on the production techniques and nutritional properties of LPC (Gerloff et al., 1965; Huang et al., 1971; Lu and Kinsella, 1972; Betschart, 1974; Edwards et al., 1975; Wang and Kinsella, 1976; Anelli et al., 1977; Bray et al., 1978; Gwiazda and Saio, 1981; Nazir and Shah, 1987). The high pigment content in LPC has been one of the major obstacles to its utilization in the human diet. For this reason the extraction and recovery of carotenoids should not only improve the sensory characteristics of LPC, but also generate a valuable by-product that could add economical value to the production of LPC.

The solvent properties of supercritical CO_2 (SC–CO_2) and its application to the extraction of many natural products have been the subject of many reviews (Paul and Wise, 1971; Bott, 1980; Schneider et al., 1980; Brogle, 1982; McHugh and Krukonis, 1986; Rizvi et al., 1986). Among the novel applications of supercritical fluid extraction (SCFE) are the recovery of aromas from spices, processing of hops, decaffeination of coffee, and extraction of lipids (Hubert and Vitzthum, 1978; Sharpe and Crabb, 1980; Stahl et al., 1980; Zosel, 1981; Vollbrecht, 1982; Friedrich, 1984). To date there have been only a few citations in the literature concerned with the extraction and recovery of natural pigments by SCFE (Haeffner and Coenen, 1986; Yamaguchi et al., 1986; Manabe et al., 1987).

Carotenoids are a group of pigments that can easily degrade from exposure to heat and light. The use of SCFE with CO_2 offers potentially milder extraction conditions than those utilized in traditional extraction methodology. For example, the high temperatures (130°C) applied during the extraction of annatto causes isomerization and degradation of the bixin molecule, thus affecting its pigmentary properties (Preston and Rickard, 1980). The object of our research was to investigate the possibility of extracting specific carotenoids (carotene and lutein) from a natural product, using SC-CO_2. Such experiments also provide additional information on the solvent power of SC-CO_2 towards compounds that have subtle differences in molecular structure and weight.

MATERIALS & METHODS

LPC preparation

LPC was prepared from alfalfa (Medicago sativa L.) harvested during the preflowering stage. After washing in a 0.1% sodium metabisulfite solution, the grass was chopped and squeezed according to a described pilot plant method (Fiorentini and Galoppini, 1981), and the collected juice was rapidly centrifuged (4200 × g, retention time 15 sec) to separate any remaining fiber particles (Lencioni et al., 1984). The juice was then heated to 85-90°C by steam injection to obtain a protein coagulum that was recovered by centrifugation (2000 × g, retention time 5 min). The end product of this operation was subsequently freeze-dried.

Extractions

The extractions were performed with the apparatus shown in Fig. 1. Commercial grade CO_2 is contained in a cylinder (A) and the pressure is monitored at a gauge (TP). Before reaching the two-stage, air-driven booster compressor (C) (mod. AGT-62/152-C, Haskel Eng.

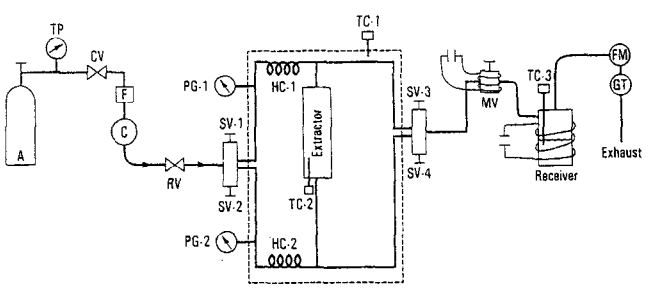

Fig. 1—Supercritical fluid extraction system (see text for description). Dashed lines indicate thermostated region.

Authors King, Friedrich, and Eskins are with the USDA-ARS-NRRC, 1815 N. University St., Peoria, IL 61604. Author Favati is with Istituto di Industrie Agrarie dell 'Universita', 56100 Pisa, Italy.

Corp., Burbank, CA), the gas flows through a check valve (CV) and a 5 μ particulate filter (F) (Norman Eq. Co., Bridgeview, IL). The CO_2 pressure is set to the desired value by adjusting to air intake valve to the compressor and is kept steady by utilizing a relief valve (RV). By subsequently opening and closing valves SV1, 2, 3, and 4, the gas can be allowed to flow in either direction through the extractor vessel, which is vertically placed in an oven. After entering the thermally controlled region, the gas flows through a 3-meter coil in length (either HC 1 or 2) to assure thermal equilibrium before reaching the extractor. The extractor consists of a 316 SS tube, pressure rated to 76 MPa at room temperature, with dimensions of 1.75 cm i.d. × 55.9 cm. Pressure drops in the extractor could be detected by observation of the inlet and outlet pressure gauges (PG 1 and 2).

The gas leaving the extractor flows to the glass receiver vessel through a micrometering valve (MV), used for adjusting the exit flow to the desired value. The receiver consists of two main parts, a modified connecting vacuum joint and a round-bottom flask of adequate volume. The exit line from the micrometering valve fits into the vacuum joint through a teflon adaptor that prevents any leakage of the solute-laden gas. Due to the sudden pressure drop that occurs in the receiver, and the subsequent rapid cooling of the solute-laden gas stream, both the MV and the receiver are electrically heated to prevent clogging of the exit line. The CO_2 and the extract separate in the receiver and the gas passes through a flow meter (FM) and a gas totalizer (GT) before being vented to the atmosphere. The temperature of the thermostatic chamber, extractor and receiver are monitored by thermocouples (TC 1, 2, and 3).

The extractions were run on 45–50 g samples of LPC at a temperature of 40°C and pressures of 10, 30, 50 and 70 MPa. Recorded flow rates were in the range 5–6 L/min at ambient conditions, and a standard amount of 3,000L of CO_2 (equivalent to 5,400g) was used in each test. The material extracted was collected at 400, 800, 1200, 1600, 2000, and 3,000L intervals of CO_2 and analyzed afterward for carotenoid content.

Analysis

The proximate compositions of LPC samples before and after the extraction were determined according to standard AOAC (1984) methods (moisture 7.007; ash 7.009; crude protein 7.015; crude fat 7.061). Pigments were extracted from the LPC according to the method of Eskins and Dutton (1979), while pigments extracted by the SC-CO_2 were dissolved in acetone:hexane (50:50 v/v) solution. Chromatographic analysis of the extracts was performed with a slight modification of the method of Eskins and Harris (1981). The pigments were separated on a μ-Bondapak C-18 column (Waters Associates, Milford, MA) with methanol:water (80:20 v/v - slovent A) and ethyl acetate (solvent B) as eluents. A linear gradient was utilized for going from 100% A to 75% B in 30 min and was then held isocratically for 15 min. The solvent flow was set at 1.0 mL/min, with detection performed at 436 nm. Peak identification was achieved through the following method: by comparing their retention time to that of injected standards, by spiking the extracts with known standards and by taking their absorption spectra during elution from the column. Carotenoids were quantitated by comparing the areas of the peaks with those obtained by injecting standards of known concentration.

RESULTS & DISCUSSION

EXTRACTION of a natural product via SCFE requires optimization of the extraction conditions and characterization of the residual product. In this study, the rate of carotenoid extraction was established by analyzing extracts taken at discrete intervals over an extended extraction time. From this information carotenoid solubilities in SC–CO_2 were calculated as a function of pressure. This provided a measure of the extraction specificity for a particular component. Complementary analysis of residual LPC verified the results obtained on the extracts, as well as potential changes in the product remaining in the extractor.

Figures 2 and 3 show the recoveries for carotene and lutein obtained at different extraction pressures as a function of the total liters of gas passed through the extractor vessel. When the extractor was operated at 10 MPa, there was a small uptake of the carotene component into the dense gas phase, which did not appreciably increase with respect to time. Increasing the

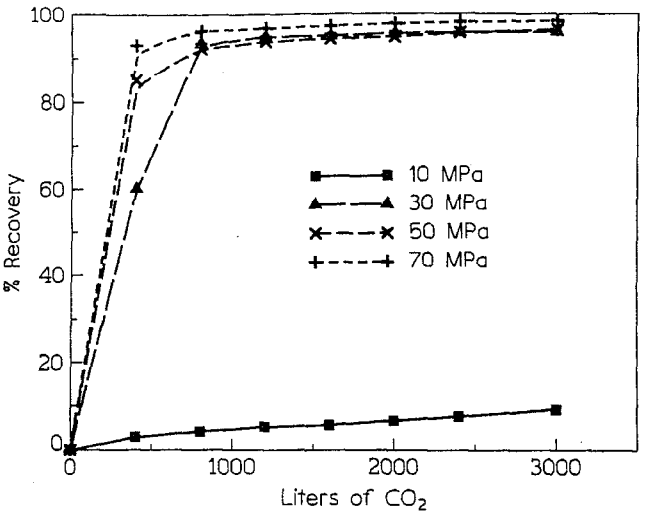

Fig. 2—Effect of pressure on the extraction of carotene at 40°C as function of total CO_2 used.

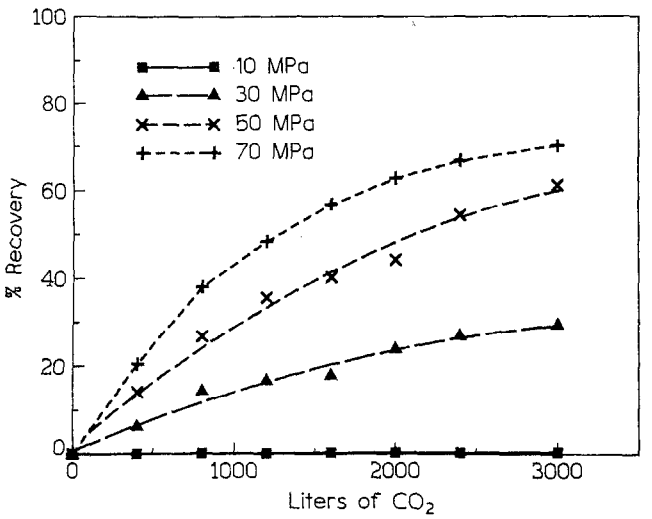

Fig. 3—Effect of pressure on the extraction of lutein at 40°C as function of total CO_2 used.

gas pressure to 30 MPa resulted in a significant increase in the amount of carotene extracted with respect to total gas volume passed through the extractor. At this level of gas compression, there was over a tenfold increase in the amount of carotene removed from the LPC. Operation of the extraction system at even higher pressures (50 and 70 MPa) also resulted in the rapid removal of carotene from the LPC, the recovery being completed after only 800 L of carbon dioxide had passed through the extractor bed.

Results for lutein removal from the LPC are in sharp contrast to those cited for carotene. The amount of lutein removed at 10 MPa is negligible, even after passage of 3000L of compressed CO_2 (Table 1). Elevation of the pressure to 30 MPa resulted in an enhancement of lutein concentration in the gas phase, reaching 29% of the theoretical yield after passage of 3000 L of CO_2. Even larger yields of lutein were recorded when the gas pressure was increased to 50 and 70 MPa. However, the rate of increase in lutein recovery with CO_2 passage was gradual, asymptotically approaching values of 61% and 70% recovery, respectively, for the above compression levels. The rapid increase in carotene solubility early in the extraction experiments indicated that most of the available carotene could be readily removed in approximately 3 hr. The extraction results suggested that a fractionation of carotene from lutein could

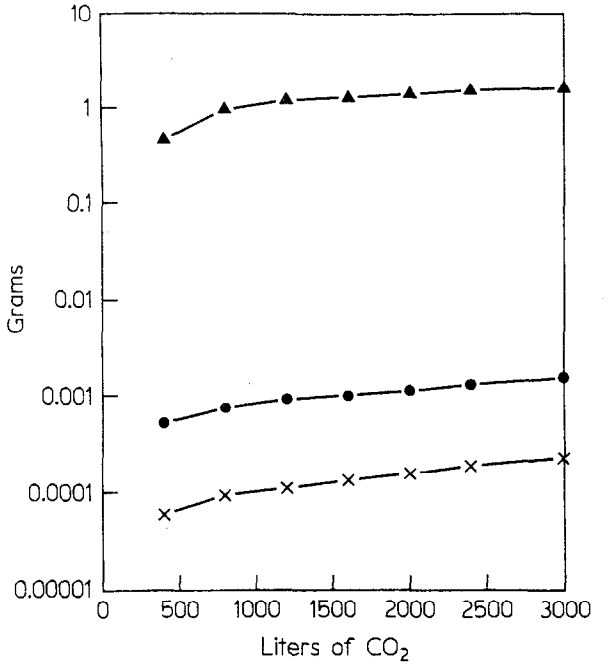

Fig. 4—Weight of the total extract (▲—▲), carotene (●—●), and lutein (x—x) collected at 10 MPa vs liters of extraction gas

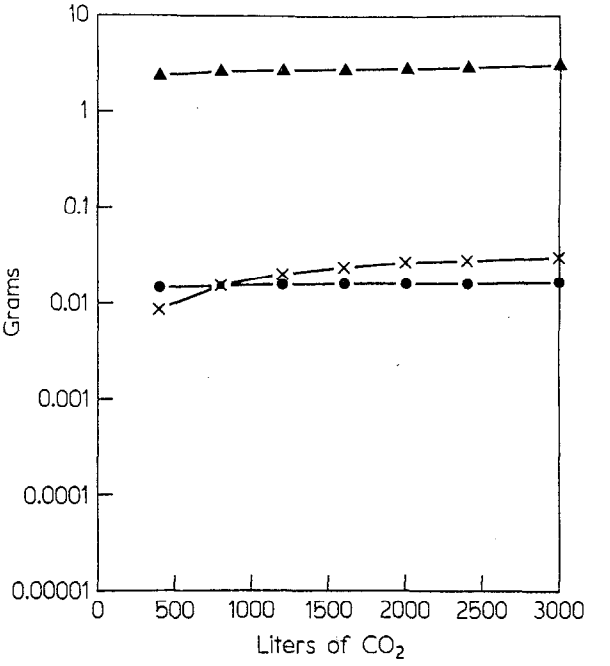

Fig. 5—Weight of the total extract (▲—▲), carotene (●—●), and lutein (x—x) collected at 70 MPa vs liters of extraction gas.

Table 1—Proximate composition of untreated and residual LPC (dry basis) and carotenoid recovery at different extraction pressures

	Untreated LPC	Extraction pressure			
		10 MPa	30 MPa	50 MPa	70 MPa
Ash (%)	12.8	13.2	14.1	14.2	13.9
Crude fat (%)	13.00	7.46	6.50	6.22	5.39
Crude protein (N × 6.25) (%)	46.19	48.00	50.63	51.13	51.25
Carotene extracted (%)	–	9.19	95.98	96.47	98.48
Lutein extracted (%)	–	0.52	29.52	61.23	70.20

Table 2—Reduce density (ρ_r) and solubility parameter (δ) values for CO_2 at 40°C and different pressures

Pressure	ρ_r	δ ($cal^{1/2}/cm^{3/2}$)
10 MPa	1.363	5.57
30 MPa	1.940	7.92
50 MPa	2.119	8.66
70 MPa	2.223	9.12

be obtained in the first stage of the extraction process, especially at 30 MPa, where the weight of carotene extracted was approximately four times greater than that of lutein.

It should be emphasized that considerable extraneous material was also removed from the LPC during the course of the supercritical fluid extraction. The total amount of material extracted at 10 MPa was 3.44% of the dried LPC, while 6.46% was obtained at 70 MPa. The percentage of a specific carotenoid extracted during any of the experimental runs was much lower as shown by the results presented in Fig. 4 and 5. Here the total extract weight and the individual weights of extracted carotene and lutein are plotted versus the total liters of gas passed through the LPC. At the 10 MPa extraction pressure (Fig.4), there is approximately a thousand-fold difference in the level of total extractives versus the extracted carotene, and this difference becomes almost ten times larger for the extracted lutein. Enrichment of the carotenoid content in the total extract occurs at higher extraction pressures as shown in Fig. 5, where the level of these carotenoids has increased to approximately 1.5% of the total extract. The presence of these contaminants should not be viewed as a serious deterrent to the industrial use of such an extract, since many carotenoid preparations are sold in a lipophilic base. In addition, it may be possible to remove the carotenoids from these extraneous contaminants by an alteration in SCFE conditions.

A crude characterization of the residual proteinaceous matter left after extraction at the designated pressures was also performed (Table 1). The ash content appeared to increase slightly over the value obtained on the unextracted LPC. This was probably due to the removal of organic components whose volatility was enhanced in the presence of the dense gas. There was an appreciable drop in the fat content of the LPC over that found in the original sample. This is in concurrence with literature citations on the solubility of triglycerides and oils in supercritical CO_2 (Friedrich and List, 1982; Friedrich et al., 1982; Stahl et al., 1982; Stahl et al., 1984). The removal of these lipid components conversely affected the protein level of the residual product, raising the crude protein from the original 46.19% to 51.25% after extraction at 70 MPa. Carotenoids left in LPC are in agreement with the results presented in Fig. 2 and 3 and are expressed as percent of total available lutein and carotene extracted.

A rationalization of the above solubility trends and extraction results can be performed with the aid of solubility parameter theory. As shown in Table 2, the reduced density (ρ_r) of the Sc-CO_2 increases with pressure, hence the solubility parameter (δ) of the solvent can be calculated from the equation proposed by Giddings et al. (1968) as

$$\delta_{gas} = 1.25\ P^{1/2}\ (\rho_{r,gas}/\rho_{r,L}) \qquad (1)$$

where P_c = critical pressure of the gas; $\rho_{r,gas}$ = reduced density of the gas; $\rho_{r,L}$ = reduced density of the gas at infinite compression.

The resultant solubility parameters for CO_2 are also tabulated in Table 2, increasing from a value of 5.6 $cal^{1/2}/cm^{3/2}$ at slightly above the critical pressure of CO_2 to 9.1 $cal^{1/2}/cm^{3/2}$ at the pressure of 70 Mpa.

Solubility parameter theory predicts that the maximum solubility of a solute is attained when the solubility parameters of the solvent and solute are equal. An approximation of the solubility parameters of such complex compounds as lutein and

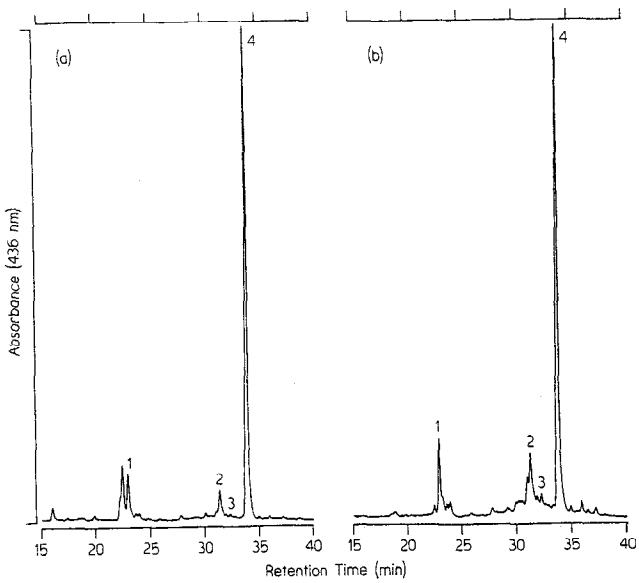

Fig. 6— HPLC chromatograms of the first (a)and the last (b)fraction collected during SCFE at 10 MPa. (Lutein; (2) pheophytin b; (3) pheophytin a; (4) carotene

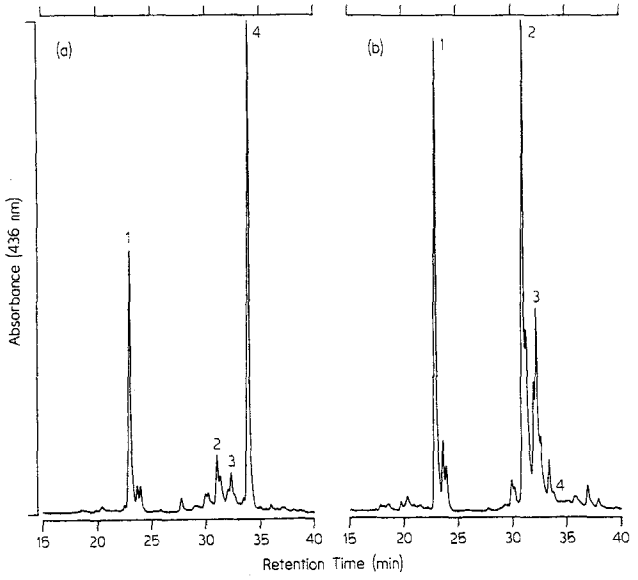

Fig. 7—HPLC chromatograms of the first (a) and the last (b) fraction collected during SCFE at 70 MPa. (1) Lutein; (2) pheophytin b; (3) pheophytin a: (4) carotene.

carotene is best accomplished by the group contribution method, in which specific energy and volume increments are assigned to individual structural units comprising the molecular structure (Fedors, 1974). Summation of these individual group contributions for the above carotenoids yielded solubility parameters of 8.71 $cal^{1/2}/cm^{3/2}$ for carotene and 10.01 $cal^{1/2}/cm^{3/2}$ for lutein. Examination of the results in Table 1 for carotene extracted at various pressures, in conjunction with the solubility parameters for CO_2 in Table 2, indicates that maximum extraction of carotene is attained at a gas solubility parameter between 8.6–9.0 $cal^{1/2}/cm^{3/2}$. This is very close to the solubility parameter of carotene, and hence in accord with the basic tenets of the solubility parameter theory. Likewise, the higher solubility parameter of lutein predicts that higher gas compression levels will be required to maximize the extraction of this carotenoid. The extraction data given in Table 1 support this hypothesis, and calculations indicate that CO_2 pressures in excess of 140 MPa would be required to give the gas a cohesional energy density equivalent to that exhibited by lutein. Further quantitative application of the above theory is not warranted, because the quoted extraction results are not obtained on neat carotenoids. The amount of carotene extracted at the highest pressures in the described study is ultimately limited by its concentration in the natural substrate (LPC) and not by the solute capacity of the extraction gas. Results reported on carotene solubility in liquefied CO_2 (Hyatt, 1984) support the latter conclusion.

Figures 6 and 7 represent typical HPLC chromatograms obtained from the analysis of the first and last fractions collected during the 10 and 70 MPa extractions. As previously emphasized, the extent of carotenoid extraction at 10 MPa was very low, and the HPLC results showed that the ratio between the carotene and lutein peaks was similar over the seven fractions collected. At an extraction pressure of 70 MPa, there was a drastic change in the relative amount of the two carotenoids. At this pressure, carotene remained the major pigment extracted in the first fraction; however, the lutein content was also substantially increased in this sample. The ratio of these carotenoids was reversed in the last fraction collected, as indicated in Fig. 7 (b). The HPLC analysis also indicated that there was coextraction of green pigments, mainly pheophytin a and b.

The presence of pheophytins and the absence of significant levels of chlorophyll a and b were confirmed from HPLC analysis of the untreated LPC. Furthermore, Bickoff et al (1954) reported that the total amount of violaxanthin and neoxanthin represented over 50% of the total xanthophylls found in fresh alfalfa. However, no significant amounts of these carotenoids were found in the untreated LPC. These major changes in pigment content and composition are related to the production of the LPC by heat coagulation. It should be noted that the detection of green pigments in the extracts obtained at 10 MPa was possible only by HPLC analysis, since the resultant extracts were yellow/orange in appearance to the human eye. At higher extraction pressures, the presence of green pigments could be visually perceived only in extracts containing a minor amount of carotene.

In conclusion, the above results have demonstrated the feasibility of obtaining extracts enriched in specific carotenoids by SCFE. Such natural colorants could potentially be used directly in food compounding processes since there were no solvent residuals in the supercritical fluid extracted product. Analytical results obtained on the residual protein left after supercritical fluid processing suggested that treatment with dense carbon dioxide had not affected the nutritional potential of this protein supplement. The combination of these two factors supports wider application of SCFE for the processing of value-added products, while obtaining a commodity additive for incorporation into foodstuffs.

REFERENCES

Anelli, G., Fiorentini, R. Massignan, L., and Galoppini, C. 1977. The polyprotein process: a new method for obtaining leaf protein concentrates. J. Food Sci. 42: 1401.

AOAC. 1984. "Official Methods of Analysis," 14th ed. Association of Official Analytical Chemists, Washington, DC.

Betshart, A. 1974. Nitrogen solubility of alfalfa protein concentrates as influenced by various factors. J. Food Sci. 39: 1110.

Bickoff, E. M., Livingston, A. L., Bailey, G. F., and Thompson, C. R. 1954. Xanthophylls is fresh and dehydrated alfalfa. J. Agric. Food Chem. 2: 563.

Bott, T. R. 1980. Supercritical gas extraction. Chem. Ind. 15: 288.

Bray, W. J., Humphries, C., and Ineritei, M. S. 1978. The use of solvents to decolorize leaf protein concentrate. J. Sci. Food Agric. 29: 165.

Brogle, J. 1982. CO_2 as a solvent: its properties and applications. Chem Ind. 19: 385.

Cutler, R. G. 1984. Carotenoids and retinol: their possible importance in determining longevity in primate species. Proc. Natl. Acad. Sci. 81: 7227.

Edwards, R. H., Miller, R. E., de Fremery, D., Knukles, B. E., Bickoff, E. M., and Kohler, G. O. 1975. Pilot plant production of an edible white fraction leaf protein concentrates from alfalfa. J. Agric. Food Chem. 4: 621.

Eskins, K. and Dutton, H. J. 1979. Sample preparation for high-perform-

ance liquid chromatography on higher plant pigments. Anal. Chem. 51: 1885.
Eskins, K. and Harris, L. 1981. High-performance liquid chromatography of etioplast pigments in red kidney bean leaves. Photochem. Photobiol. 33: 131.
Fedors, R. F. 1974. A method for estimating both the solubility parameters and molar volumes of liquids. Polymer Eng. Sci. 14(2): 147.
Fiorentini, R. and Galoppini, C. 1981. Pilot plant production of an edible alfalfa protein concentrate. J. Food. Sci. 46: 1514.
Friedrich, J. P. 1984. Supercritical CO_2 extraction of lipids from lipid-containing materials. U.S. patent 4,466,923, August 21.
Friedrich, J. P. and List, G. R. 1982. Characterization of soybean oil extracted by supercritical carbon dioxide and hexane. J. Agric. Food Chem. 30: 192.
Friedrich, J. P., List, G. R., and Heakin, A. J. 1982. Petroleum-free extraction of oil from soybeans with supercritical CO_2. J. Am. Oil Chem. Soc. 59: 288.
Gerloff, E. D., Lima, I. H., and Stahmann, M. A. 1965. Amino acid composition of leaf protein concentrate. J. Agric. Food Chem. 13:139.
Giddings, J. C., Myers, M. N., McLaren, L., and Keller, R. A. 1968. High pressure gas chromatography on nonvolatile species. Science 162: 67.
Gwiazda, S. and Saio, K. 1981. Preparation of white leaf protein concentrates using calcium salts. Agric. Biol. Chem. 45: 2659.
Haeffner, E. A. and Coenen, H. F. 1986. Color extraction from paprika using supercritical gases. Paper No. 105, presented at 191st ACS, National Meeting, New York, NY, April 13–18.
Huang, K.H., Tao, M.C., Boulet, M., Riel, R.R., Julien, J.P., and Brisson, G.J. 1971. A process for the preparation of leaf concentrates based on the treatment of leaf juices with polar solvents. J. Inst. Can Technol. Aliment. 4(3): 85.
Hubert, P. and Vitzthum, O.G. 1978. Fluid extraction of hops, spices, and tobacco with supercritical gases. Angew. Chem. (Int. Ed. Engl.) 17: 710.
Hyatt, J.A. 1984. Liquid and supercritical carbon dioxide as organic solvents. J. Org. Chem. 49: 5097.
Lencioni, L., Fiorentini, R., and Galoppini, C. 1984. Caseificazione di latte di pecora miscelato con succo di erba medica. Industrie Alimentari 23: 106.
Lu, P. and Kinsella, J.E. 1972. Extractability and properties of protein from alfalfa leaf meal. J. Food Sci. 37: 94.
Manabe, A., Tokumori, T., Sumida, Y., Yoshida, T., Hatano, T., Yazaki, K., and Okuda, T. 1987. Application of supercritical fluid extraction to components of crude drugs and plants. III. Extraction of pigments from lithospermum root and licorice root. Yakugaku Zasshi 107(7): 506.
McHugh,M.A. and Krukonis, V.J. 1986. "Supercritical Fluid Extraction", Butterworths, Stoneham, MA.
Nazir, M. and Shah, F.H. 1987. Studies on Persian clover (Trifolium resupinatum). Part III: effect of different methods of drying on the nutritive value of leaf protein concentrate. Plant Foods Hum. Nutr. 37: 3.
Paul, P.F.M. and Wise, W.S. 1971. "The Principle of Gas Extraction". Mills & Boon,.London, England.
Preston, H.D. and Richard, M.D. 1980. Extraction and chemistry of annatto. Food Chem. 5(1): 47.
Rettura, G., Stratford, F., Levenson, S.M., and Seifter, E. 1982. Prophylactic and therapeutic actions of supplemental B-carotene in mice inoculated with C3HBA adenocarcinoma cells: lack of therapeutic action of supplemental ascorbic acid. J. Nat Cancer Inst. 69: 73.
Rizvi, S.S.H., Daniels, J.A., Benado, A.L., and Zollweg, J.A. 1986. Supercritical fluid extraction: operating principles and food applications. Food Technol. 40(7): 57.
Schneider, G.M., Stahl, E., and Wilke, G. (Ed.). 1980. "Extraction with Supercritical Gases". Verlag Chemie, Weinheim, West Germany.
Seifter, E., Rettura, G. Padawer, J., and Levenson, S. 1982. Moloney murine sarcoma virus tumors in CBA/J mice: chemopreventive and chemotherapeutic actions of supplemental B-carotene. J. Nat Cancer Inst. 68: 835.
Seifter, E., Rettura, G., Padawer, J., Stratford, F., Goodwin, P., and Levenson, S.M. 1983. Regression of C3HBA mouse tumor due to X-ray therapy combined with supplemental B-carotene or vitamin A. J. Nat. Cancer Inst. 71: 409.
Sharpe, F.R. and Crabb, D. 1980. Pilot plant extraction of hops with liquid carbon dioxide and the use of these extracts in pilot and production scale brewing. J. Inst. Brew. 86: 60.
Stahl, E. 1982. Extraction of natural substances by compressed gases. Fette Seifen Anstr. 84: 444.
Stahl, E., Quirin, K.W., and Blagrove, R.J. 1984. Extraction of seed oils with supercritical carbon dioxide: effect on residual proteins. J. Agric. Food Chem. 32: 938.
Stahl, E., Schutz, E., and Mangold, H.K. 1980. Extraction of seed oils with liquid and supercritical carbon dioxide. J. Agric. Food Chem. 28:1153.
Vollbrecht, R. 1982. Extraction of hops with supercritical CO_2. Chem. Ind. 12: 397.
Wang, J.C. and Kinsella, J.E. 1976. Functional properties of novel proteins: Alfalfa leaf protein. J. Food Sci. 14: 286.
Yamaguchi, K., Murakami, M., Nakano, H., Konosu, S., Kokura, T., Yamamoto, H., Kosaka, M., and Hata, K. 1986. Supercritical carbon dioxide extraction of oils from antarctic krill. J. Agric. Food Chem. 34: 904.
Zosel, K. 1981. Process for the decaffeination of coffee. U.S. patent 4,247,570, January 27.
Ms received 1/25/88; revised 5/8/88; accepted 5/12/88.

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.